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SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/486,409	06/07/95	WA-L	G [illegible]

EXAMINER
LOW, C

ART UNIT	PAPER NUMBER
1804	7

18N2/0520

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DATE MAILED: 05/20/96

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☒ Responsive to communication filed on 7 June 1995 ☐ This action is made final.

A shortened statutory period for response to this action is set to expire three (3) month(s), 0 days from the date of this letter. Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☒ Notice of References Cited by Examiner, PTO-892.
2. ☒ Notice of Draftsman's Patent Drawing Review, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____.

Part II SUMMARY OF ACTION

1. ☒ Claims 1-18 and 29-41 are pending in the application.
   Of the above, claims none are withdrawn from consideration.
2. ☒ Claims 19-28 and 42-59 have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☒ Claims 1-18 and 29-41 are rejected.
5. ☐ Claims _____ are objected to.
6. ☐ Claims _____ are subject to restriction or election requirement.
7. ☒ This application has been filed with ~~informal~~ drawings ~~under 37 C.F.R. 1.85~~ which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice of Draftsman's Patent Drawing Review, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____. has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed _____, has been ☐ approved; ☐ disapproved (see explanation).
12. ☐ Acknowledgement is made of the claim for priority under 35 U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____ ; filed on _____.
13. ☐ Since this application apppears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

In the paper filed 7 June 1995, it is noted that claims 19-28 and 42-59 have been canceled. Consequently, claims 1-18 and 29-41 are pending to which the following are applicable.

The application should be reviewed for errors.

35 U.S.C. 101 reads as follows:

> "Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter or any new and useful improvement thereof, may obtain a patent therefore, subject to the conditions and requirements of this title".

Claims 29-34 are rejected under 35 U.S.C. 101 because the claimed invention is directed to non-statutory subject matter. Insofar as the claims do not contain a recitation of a "isolated mammalian cell line" the terminology of "A mammalian cell" contains no qualifiers that exclude an intact mammal and any mammal includes within its scope a human being into which DNA containing the FLP site has been inserted. A human contains mammalian cells and human cells containing the inserted DNA for the FLP sites in a human is a claim including within its scope a human being. It is not considered patentable subject matter. The limited but exclusive property right in/to a human being is barred by the United States Constitution. See 1077 OG 24.

Claims 1-18 and 29-41 are rejected under 35 U.S.C. 112, first paragraph, as the specification does not contain a written description of the claimed invention, in that the disclosure does not reasonably convey to one skilled in the relevant art that the inventor(s) had possession of the claimed invention at the time the application was filed as set forth below.

Claims 1-18 and 29-41 are also rejected under 35 U.S.C. 112, first paragraph, because the specification does nor enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention as is presently claimed as set forth below.

Each of the above rejections are applicable because claim 1 requires the first DNA to contain a preexisting FLP site and neither the claim nor the specification indicate how the first FLP site is inserted into the genome of the cell or the animal nor how the FLP recombinase (an enzyme) is operative as

part of the "system" which is the DNA encoding the FLP recombinase and the FRT site. It is not readily apparent from the present written description as for example in mice how the recombination is effected in the mouse when the recombinase (i.e., the protein alone) is administered.

The host organism (or host or subject or said subject or said host mammal) is not described in the specification nor set forth in the claims as to any particular phenotypic characteristics effected by the FLP site(s) and the remining inserted heterologous DNA. There is no example descriptive of the process defined by claims 15-18. Here, the written description at pages 1-2 creates doubt as it indicates that manipulation is impaired due to inability to control the site of integration (i.e., how does one control the site of the first integration event?), number of copies, temporal expression, and the like. Given these difficulties, how is the mammal or the host or the subject of the process made so that the FLP target site is not at some random location within the genome and/or a gene? What gene is the site randomly located in? Before a specific gene can be targeted by DNA which contains the FLP recombination target site (FRT), it must have a specific site for recombination already in the genome and specific gene of the host. Here, the specification has not indicated a particular host or subject or said host mammal where the location of the FLP sites are initially at specific sites predetermined by the user. The present application does not disclose how to achieve these ends. Note that page 9, lines 10-14 refer to the nonhuman transgenic animals with the FLP site but does not indicate how the specificity of the placement of the site in the genome is predetermined or produced. How is the FLP site for the animal specifically targeted to a specific gene and what portions of the gene are best suited to or for integration of the FLP recombination site? Insofar as nonhuman animals contain *per se*, cells, and the FLP site is in the cells, the specification has not provided an enabling written description for site specific integration of DNA coding for the FLP site (i.e., the target for the DNA which recombines at the FLP site). How and where is the written description of inserting the first of the sites?

Precise genomic targeting requires not only precise targeting of the initial insert DNA but that the location to which it is to be inserted also be precisely identified. Absent such teaching, it cannot be said that the DNA to be targeted to the first preexisting site is precisely targeted to some known location on a DNA when that location on the DNA is unspecified. Since the integrating DNA recombines with the FRT site, but where the location of the preexisting site is unspecified, there is no

precise targeting of the integrating DNA to any specified location in the genome; i.e., the problem of inability to control the initial location of integration of the initial FRT site remains as the specification does not disclose how the initial FRT is integrated at predetermined sites on a chromosome. Thus, the disclosure is not enabled for precisely targeting the DNA to a predetermined site of that integration, i.e., the first FLP recombination target site is not precisely predetermined by its own location in the genome of the cell nor does it precisely determine its own first location in the genome. From the present specification, there is no disclosure of how this is accomplished. How does the first FLP site become specifically and precisely integrated into the genome? Note that the first full paragraph of present specification page 12 does not detail how this is done and is subject to the inability to control the site of integration referred to at present specification page 1 and as indicated in the present brief at page 6 (see the "Integration of the initial FLP recombination site is not targeted". Here, where the specification does not disclose how this is done, it would have required undue experimentation on the part of one skilled in the art to have used the present application disclosure to precisely place the first FLP target recombination site at a prespecified location defined at least by chromosome number and specific sequence of bases where there are no other inadvertent sites where the first FLP recombination target site becomes integrated into the genome. Note that the present claims call for the first FLP target recombination site to be precisely placed else the precise location of the integrating DNA is not definable.

As to the multiple FRT sites and recombination thereof, when the first DNA segment has a part of the gene and embedded in the middle of the segment, it is not clear how or what functional gene is obtained via recombination at the FRT sites. For example, where

Segment 1 is bases 1-31-FRT site -bases 32-45
Segment 2 is bases 46-66-FRT site-bases 67-100
Segment 3 is FRT site-bases 101-200-FRT site-bases 201-900

and the segments are joined 1 to 2 to 3, it is not clear that bases 1-31 would not be joined to bases 201-900 and thus, leave out bases 32-200 or that bases 46-66 would not be joined to bases 32-45 via the action of the FLP recombinase. What is the functional gene obtained? When for example, the segments are joined 1 to 3 to 2, what functional gene is obtained post recombination as for example, when the segments encode a marker gene that is silent due to the FRT sites but after recombination is

missing bases 201-900 via the action of the recombinase (note that the present application written description indicates that function can also be lost)?

The present application written description (page 9, lines 10-14) does not describe nor enable all or any non-human mammals nor any processes as applied to intact multicellular animals even where page 15 indicates humans are "contemplated". Neither page 9 nor page 15 disclose or teach how to make such an animal. Attention is also directed to the preceding paragraphs. Note that the paragraph bridging pages 15-16 starts off by "once FLP recombinase ... has been introduced into suitable host cells/organisms ..." but does not indicate how the first integrative event of inserting the first FLP site into the genome was accomplished nor does it disclose or teach how that first integrative event is specific to a given locus. Moreover, the indication of contemplated (written description at page 15) is not an enabling written description of even what characteristics an animal or nonhuman mammal would or would not have had by such loss or gain of a DNA. Nor is it apparent from the present written description that the claimed animal had been made via the present application written description. The present application written description does not demonstrate nor disclose how to obtain or that, for example, a transgenic platypus (an egg laying mammal) had been obtained or that a mule (the sterile hybrid of a male ass and an female horse) had been made with the FRT site. How is the DNA inserted into the egg of a platypus? Insofar as the claims are directed to DNA, cells, and transgenic non human animals, the Wall (Theriogenology) reference (published after applicant's filing date) presents doubt that transgene expression and the physical consequences of the transgene product are always accurately predicted (see page 62) and that the only approach that yields truly informative data is testing the transgenes in the livestock (i.e., the animals) species of interest. Here, the Houdebine (J. Biotechnol.) presents additional doubts since at page 277 it is indicates that for the great majority of cases, transgene expression is heavily dependent upon the site of integration (but in the present application the site of integration of the original or first FLP site put into the genome is at best random and appears from the present application written description to not be disclosed how the first FLP site is integrated). Moreover, the construction of the appropriate vectors is still a problem (see page 281).

Where the above are indicative of the absence of disclosure, it is apparent that an undue quantity of experimentation is needed since the application does not demonstrate the animals nor show that the FLP sites are integrated into the genome at an *a priori* preselected and predetermined genomic locus. Thus, from the present application written description there is an insufficient amount of direction and/or guidance presented as the working examples do not demonstrate the mice or any other transgenic animal nor do the examples reproducibly show gain and/or loss of function nor for instance does example 2 appear to demonstrate the gain of function when the FRT as discussed above is in the middle of a sequence. The specification does not appear to show or demonstrate regain of function post loss of function via the recombinase. Note that it is unpredictable that when the site of genomic insertion is not reproducible or known, the expression of the inserted DNA is as indicated above is dependent upon the site of integration and speaks to the unpredictability aspect since the present application admits of the impaired efficacy of control of the site of integration (page 1) and especially where present specification page 5 indicates using promoters that are transiently active in development. Thus, it is unclear as to whether or not expression from recombined promoters and/or genes that are, for example, only expressed in the adult animal would have been expressed during development of the fetal animal. As discussed in the above paragraphs, it is not readily apparent that a functional gene would have been the result of the present application written description (pages 7-8) using the above indicated DNA segments. Thus, there are factors that effect unpredictability where the contemporary knowledge of the relevant art indicates doubt on the part of others such that the claims are rejected for inadequate written description and for lack of enablement.

Claims 1-18 and 29-41 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The full spelled out words for FLP should be used else the claim is also interpretable as a phenylalanine-leucine-proline recombinase. In none of the claims is there an indication of any particular phenotypic characteristics effected by the FLP site(s) and the remining inserted heterologous DNA. In claim 2, it is not clear from the claim what are or are not the metes and bounds of "a portion of" (as well as all claims indicating "portion") in regard to a first gene or a second gene. Are they the same or different sequences for the "a portion"? Claim 2 is also indefinite since claim 1 lacks antecedent basis for "said first gene of interest" (claim 2, (iii), item (a) as well as claim 3).

In claim 2 (as well as claims 34 and 41), what is the portion of the genes that are the portions of interest? In claim 2 (as well as claims 34 and 41) is or is not the "a second DNA" the same or different from "a second gene of interest"? As to claim 2, it is unclear as to what is the function of the "functional" gene that is recited (nor is it clear in claim 5, what it is or is not that the hybrid functional gene is or is not nor what function it encodes). In claim 3 it is not clear what is or is not the additional portion of said first gene of interest that is referred to in the claim. In claim 4, it is not clear what is or is not the "a least a portion of a second gene of interest" insofar as there is no apparent function indicated for the indicated portion". Attention is also directed to all claims reciting "portion" or variations thereof. In claim 6, when the combined DNA disrupts the function of the "said first gene of interest" (which lacks antecedent basis in claim 1) it is not clear that there is an intact function that is expressed from the said second gene.

In claims 8 and 9, it is not clear as to the metes and bounds of "derived from" nor in any other claim containing the "derived from" terminology (see claims 16 and 17 as well). How many amino acids are or can be changed before the recombinase is no longer "derived from" and does a recombinase that performs the identical function as the *Saccharomyces* recombinase but which is from a different organism "derived from" the *Saccharomyces* recombinase?

Claims 10 and 18 are indefinite as SEQ ID NO: 1 does not contain 1450 bases nor does approximately mean adding 70 bases to 1380 bases. Claims 10 and 18 are indefinite since these claims contain two periods. Note that "Sequence ID No. 1" should be written as "SEQ ID NO: 1" (without any periods).

In claims 11, 12, 32, and 39 it is not clear as to what marker feature the claims refer to that is readily analyzed nor is it clear as to what is analyzed.

Claims 13 and 14 are not clear since the orientation and operability of the recited parts is not specified. For example, is the at least one FLP site in the middle of the marker gene or is it in the middle of the bacterial origin of replication? Where is, for example, the restriction endonuclease restriction site in relation to the marker gene? In claim 14 what is the "suitable sequence" and does it

or does it not include sequences inverted in relationship to the preceding segment? Does the "in tandem" (claim 14, item c) mean that the FLP sites are directly repeated without items (b)(1) to (b)(4)?

Claim 15 is indefinite as to the "derived from" terminology - see the above discussion of claims 8 and 9. Claim 15 is also indefinite because it is not clear how a functional gene is obtained when the segments of the genes contain the "FLP" site in the middle of the segments.

Claims 33 and 40 are indefinite since it is not clear what are or are not the sequences for the "functional equivalents thereof". Note also that 37 C.F.R. 1.821(d) requires the recitation of the sequence identifier.

Claims 33 and 40 are rejected under 35 U.S.C. 112, fourth paragraph, as being of improper dependent form for failing to further limit the subject matter of a previous claim since the "or functional equivalents thereof" broadens the scope of these claims.

The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> "A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.
>
> Subject matter developed by another person, which qualifies as prior art only under subsection (f) and (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103, the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 C.F.R. 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103.

Claims 1-18 and 29-34 are rejected under 35 U.S.C. 103 as being unpatentable over Sauer (US '317) taken with Golic *et al.*, Schiestl (US '757) and Sencoff *et al.*

Sauer teaches site specific recombination of mammalian cells (col 14+) using plasmids with the DNA coding for the *cre* and *lox* (cols 1, 6-7). Where Sauer does not explicitly disclose the use of DNA coding for FLP and FRT, it would have been obvious to one of ordinary skill in the art to use DNA coding for FLP and FRT in vectors for transforming *D. melanogaster* because Golic *et al.* discloses site specific recombination in *D. melanogaster* with DNA coding for FLP and FRT (see at least pages 499 and 507) where it is indicated that FRT bearing plasmids can be directed to the site of an FRT already resident in the genome suggesting its use for germline transformation which would have resulted in a transgenic animal and further indicate that "we expect that it will work in other organisms as well" which would have motivated one of ordinary skill in the art to combine the teachings of Sauer which discloses at cols 14+, site specific recombination in mammalian cells (mouse) where the combination of the Sauer and Golic *et al.* references would have resulted in a method for site specific recombination in mammalian cells or in transgenic animals. Moreover, where both Sauer and Golic *et al.* teach that the DNA for the FLP and FRT are from yeast, Sauer teaches at col 5, mating the yeast of opposite mating types which contain the plasmids with the DNA for the FLP and FRT which is a step of introducing the cells produced by the step (i) and (ii) of claim 28 into the subject where the subject is another yeast cell and where Golic *et al.* disclose mating the flies (page 500), it is a step of introducing the cells which are the male or female gametes into the subject where the subject is the other *D. melanogaster* gamete which after fertilization becomes a transgenic fruit fly. Here, where Golic *et al.* (page 507) indicate that "we expect that it will work in other organisms as well", it is clear from the Scheistl patent to have used mammalian cells (see at least col 11) with DNA that effected recombination since the Schiestl patent at column 20+ discloses constructs containing FLP and FRT for recombination and explicitly tell one of ordinary skill in the art to use mammalian cells wherein the Senecoff *et al.* reference discloses the known sequences for FLP mediated recombination (see for example figure 1) that are discussed and disclosed in the Golic *et al.* and Schiestl references. Thus, the claimed invention was within the ordinary skill in the art to make and use at the time it was made and was a whole, clearly prima facie obvious.

Claims 35-41 are rejected under 35 U.S.C. 103 as being unpatentable over Sauer (U.S. '317) taken with Golic *et al.*, Schiestl (US '757) and Sencoff *et al.* as applied to claims 1-18 and 29-34 above, and further in view of Palmiter *et al.* (Ann. Rev. Genet.)

Sauer and Golic *et al.* are applied as indicated above and where Golic *et al.* indicates expectation of success as indicated above, one of ordinary skill in the art would have found it obvious to combine the teachings in the Palmiter *et al.* reference which discloses introduction of the transforming DNA into totipotent teratocarcinoma cells or embryonic stem cells which can be introduced into the developing embryo by aggregation of the cells (wherein the cells become the non human transgenic animal). Here, where Sauer taken with Golic *et al.* disclose the plasmids with the FLP and FRT DNA for site specific recombination, it would have been obvious to one of ordinary skill in the art given that Golic *et al.* indicate that "we expect that it will work in other organisms as well" and made transgenic animals, to modify the process by using totipotent teratocarcinoma cells or embryonic stem cells as disclosed by Palmiter *et al.* which are later aggregated with the developing mouse embryo. Thus, the claimed invention was within the ordinary skill in the art to make and use at the time it was made and was as a whole, *prima facie* obvious.

No claims are allowed.

Papers related to this application may be submitted by facsimile transmission to Group 1800 via the PTO Fax Center located in Crystal Mall 1 (CM1) and must conform to the notice published in the Official Gazette, 1096 OG 30 (15 November 1989). The telephone number assigned to Art Unit 1804 in the CM1 PTO Fax Center is (703) 308-0294.

Inquiry concerning this communication or earlier communications from the examiner should be directed to Christopher Low whose telephone number is (703) 308-2923. Inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

CSFL
15 May 1996

Christopher S. Low

CHRISTOPHER S. F. LOW
PRIMARY EXAMINER
GROUP 1800